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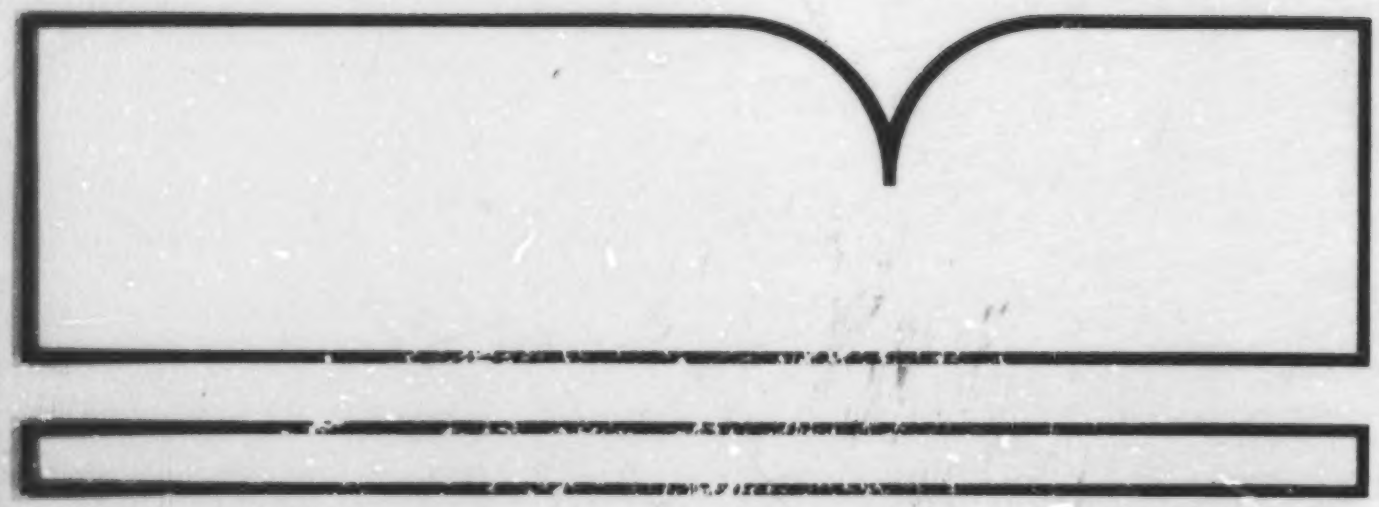

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DEVELOPMENTAL IMMUNOTOXICOLOGY

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16. ABSTRACT

This is a review of the development of the immune system and the consequences following perinatal exposure to environmental toxicants.

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Survival of mammalian species requires the presence of an intact functional immune system. The immune system is composed of myriad cellular and humoral components which are responsible for protecting the individual from invading microorganisms, eliminating foreign material, and destroying certain neoplastic cells. Evidence for the importance of a functional immune defense system comes from animal and human studies. For example, clinical studies indicate that the manipulation of the immune system in patients undergoing therapy for certain diseases or following organ transplant leads to an increased susceptibility to infectious agents and certain forms of neoplasia (1,2). Recently, the description of the disorder known as acquired immune deficiency syndrome (AIDS) has further underscored the critical role played by the immune system in survival (3). The lethality of this disorder is not due directly to the causative agent, but to sequelae of comprised immunity. A majority of AIDS patients succumb to overwhelming infection with opportunistic microorganisms and may also develop rare forms of cancer. Over the years a growing body of evidence also indicates that a variety of natural and man made environmental agents are capable of modulating the immune system (4-7).

The development of the immune system in mammals involves a precise sequence of steps which begins early in gestation. Perturbation or abrogation of this developmental sequence of events can lead to immune dysfunctions which may be life threatening. Defects in the development of the immune system due to heritable changes in the lymphoid elements have provided clinical and experimental examples of the devastating consequences of impaired immune development (8). For example, mice born with the homozygous mutation (nu/nu) are hairless or "nude" and lack a thymus (9). These mice

lack cell-mediated immune responses and are vulnerable to infection. Consequently, they do not generally survive more than a few weeks after birth unless raised in an environment that reduces exposure to pathogens.

The developing organism is in general more dramatically affected by exposure to physical or chemical agents than is the fully mature individual (10). For example, agents that normally require acute doses over a long period of time to produce cancer in adults may be capable of inducing cancer following dosing at a much lower level and over a shorter period of time during fetal development (11,12). Furthermore, the most profound effects of agents that modulate the immune system occur when dosing of the compound begins during the development of the lymphoid system (5).

Potential functional defects caused by exposure to toxic agents during development range from life threatening suppression of vital components of the immune system to altered or poorly regulated responses that can be debilitating (13). The full extent of the immunological effects resulting from perinatal exposure to environmental agents is not yet known; however, the potential for serious consequences is obvious.

The purpose of the paper is the following: 1) to establish the need to study the effects of toxic chemicals on the developing immune system; 2) to examine the consequences to health following impairment or defects in the ontogeny of the immune defense system; and 3) to identify approaches and methodologies for assessing the sensitivity of the immune system to environmental chemicals in the developing organism. In order to accomplish these goals, the discussion which follows will be divided into three sections with reference made to both human and animal studies as appropriate. The first section will be devoted to a general overview of the immune system followed by a discussion

of methodologie available for function assessment of the various components of the immune system. The second section will outline the ontogeny of the human immune system and present examples of intrinsic or heritable defects in the human immune system. The third section will be a summary of some examples of environmental agents that have been found to affect adversely the developing immune system in experimental animals.

I. OVERVIEW OF THE IMMUNE SYSTEM

A. Specific and Nonspecific Immunity

The host defense system is comprised of mechanical, cellular and humoral components which protect the body against a variety of infectious agents (i.e., bacteria, viruses, and parasites) and neoplastic diseases. Examples of the mechanical components include the skin and its mucous membranes which form a physical barrier to infectious agents, the lavaging action of tears and saliva, and the ciliary action in the respiratory tract. The self-sterilizing properties of the skin which result from the presence of fatty acids and low pH which kill certain bacteria, and the presence in tears, nasal secretions, and saliva of the enzyme lysozyme which is capable of breaking down the mucopeptide layer in the cell walls of many bacteria constitute nonspecific chemical mechanisms (14). While these factors have a role in reducing penetration by microorganisms, the traditional immune system consists of highly specialized cells and cell products which protect the host from invading microorganisms, foreign tissue and neoplastic cells. Host defense functions can be characterized as consisting of the cooperative action of specific and nonspecific cellular and humoral responses. The cellular elements of the immune system consist of lymphoid and phagocytic

cells, and the soluble mediators include specific antibodies (i.e., humoral immunity) as well as complement, interferon and lymphokines. Although antibodies are produced by cells, the distinction drawn between humoral and cellular immunity is based upon the ability to transfer passively humoral immunity to an antigenically naive recipient with cell-free, antibody-containing serum.

The phagocytic cells which are responsible for non-specific defenses against infectious agents include two leukocyte types, the neutrophilic granulocyte and the monocyte or macrophage. Neutrophils are the first cells to arrive at the site of bacterial invasion, particularly in the case of infections caused by pyogenic microorganisms. Monocytes reach the area of infection later and subsequently differentiate into macrophages which are more efficient phagocytes. Engulfment and digestion of the invading organisms by phagocytes are enhanced in the presence of specific opsonizing antibodies produced by lymphocytes. The macrophage also serves as a scavenger cell which removes dead and effete cells.

Macrophages are also important as effector cells in tumor immunity. Under certain circumstances these cells are capable of destroying neoplastic cells following "activation" by a number of substances, including lymphokines and interferon. Tumor cell destruction by "activated" macrophages is in general mediated by a cell contact-requiring mechanism rather than by phagocytosis of the target cell (15). Macrophages also participate in specific immune responses both as effector cells in cellular immunity reactions and as auxiliary cells in the induction of certain immune responses (16).

Another recently described host defense mechanism that is mediated by cell to cell contact is natural killer (NK) cell activity. This is a form

of nonspecific immunity suggested as an important component in host surveillance and destruction of tumor cells as well as in protection against certain viral and parasitic infections (17). Several in vitro studies have demonstrated that NK activity is mediated by cells (lymphocytes) that require no prior exposure to the antigen(s) of a particular malignant cell before becoming cytotoxic and can function independently of other lymphocytes (see below) which require an "education" phase (13). These cells possess certain properties which distinguish them from other cytotoxic cells (e.g., macrophages, granulocytes and cytotoxic T-lymphocytes) and have been demonstrated both in humans and other species tested (19). In addition to killing malignant cells in vitro, NK cells have been shown to inhibit the spread of cancer by circulating tumor cells in vivo (20). The level of NK cell activity is enhanced by soluble products (e.g., interferon, interleukin) of other cells active during the immune response (21).

The majority of cells that participate in specific immune responses are lymphocytes which recognize and interact with antigen molecules through receptors on their membrane surface. These cells constitute the antigen specific portion of the immune host defense system and are broadly divided into two groups, the B- and T-lymphocytes. While these cells are similar morphologically, they have different functional characteristics and can be identified by the presence of unique surface receptors and/or antigens. Both T- and B-lymphocytes are derived from stem cells in the bone marrow which then pass through various stages of development to differentiate and mature into functional cells of the immune system (22).

B lymphocytes or "bursa derived" lymphocytes are the progenitors of antibody forming cells and are responsible for humoral immune responses.

This population of lymphocytes, as first identified in the chicken, is derived from bone marrow stem cells which undergo a process of antigen-independent maturation in the bursa of Fabricius (23). No primary organ for B cell differentiation has been identified in mammals; however, part of the differentiation in these species takes place in the bone marrow. Following appropriate stimulation by antigen, B lymphocytes proliferate, undergo morphological changes, and develop into plasma cells which actively synthesize and secrete antibody. There are five major classes of antibodies, IgG, IgA, IgM, IgD and IgE, which are distinguished by their physical and chemical structure.

IgG is the most abundant immunoglobulin and participates in immunity against many infectious agents including bacteria, viruses, some protozoans and fungi. The biological activities of IgG include neutralization of toxins and viruses, agglutination or clumping of infectious agents, opsonization of microorganisms, which facilitates phagocytosis by neutrophils and macrophages, and a bacteriolytic capacity in the presence of complement. In mammals IgG is also transported across the placenta, thereby providing the fetus amd neonate with protection against infectious agents (22).

IgA is the second most abundant serum immunoblobulin in humans. Its importance to the immune system lies in the fact that it is found in external secretions with high concentrations found in the respiratory, gastrointestinal and genitourinary tracts. IgA antibodies are capable of neutralizing toxins and viruses and of agglutinating bacteria and other parasites. While IgA does not cross the placenta, it nevertheless contributes to the immunity of human newborns due to the fact that high concentrations of IgA are found in colostrum (24).

IgM, the largest immunoblobulin molecule, is found in serum at concentrations

lower than IgA. Like IgG, IgM has neutralizing, agglutinating and bacteriolytic capacity. Following antigenic challenge, IgM levels peak within a few days and then decline as increased levels of IgG are produced (25). Because of its molecular size, IgM does not cross the placenta.

IgD, a fourth immunoglobulin class, has no clear biological role. Increased levels of this antibody are seen during chronic infections and a large proportion of human B lymphocytes possess IgD on their cell membrane (22).

IgE or reagenic antibody is found only in trace amounts in serum. This immunoblobulin mediates allergic reactions of the immediate type by binding to mast cells and basophils triggering the release of vasoactive mediators such as histamine (26).

Antibody production usually requires the participation of macrophages and both B- and T-lymphocytes. Upon encountering an antigen, macrophages trap and concentrate the antigen, process it and "present" it to T cells (27). In addition, macrophages secrete a variety of products, among which are stimulators of T cell division (28). Antigen responsive T cells are generated during this process and, in turn, release other chemicals known as lymphokines which enhance or suppress the responses of other participating cells (29). In general, two subtypes of antigen-specific T cells result from cell division: (a) T cells which provide "help" to the precursors of antibody producing B cells and (b) T memory cells that revert to a long-lived quiescent stage, forming a pool of antigen-specific cells capable of rapid proliferation on re-exposure to the antigen (29). B lymphocytes respond to antigen by undergoing differentiation either to end-stage plasma cells which synthesize antibodies or to B memory cells. In other cases, cooperation

between T- and B-lymphocytes is not required for antibody production against an antigen. These are called thymus-independent antibody responses (30) and are generally elicited by polysaccharides which result primarily in the production of only IgM antibodies.

T lymphocytes, which are responsible for cellular immunity and important regulatory functions, originate in the thymus. Evidence for this comes from experiments in which removal of the thymus from neonatal mice renders the animals incapable of mounting a T lymphocyte dependent immune response (31). T lymphocytes are involved in cell-mediated immune (CMI) responses which include resistance to infection with certain viruses, facultative intracellular bacteria, helminths and some protozoans (32). T lymphocytes also are involved in contact or delayed type hypersensitivity (DTH) reactions (33), and in the rejection of foreign tissues such as transplants (allografts). They also function as regulatory cells (helper and suppressor T cells) which modulate the activities of B cells or other T cells, and can act as cytotoxic cells against tumors (22). Cytotoxic cells are sensitized T lymphocytes that are able to specifically lyse target cells to which they bind. T cells can also produce lymphokines which are capable of modulating the activity of other cells (32). In recent years, the use of sophisticated techniques has demonstrated numerous subsets of T cells which participate in the amplification or suppression of various immune responses (34,35).

In addition to B- and T-lymphocytes, which have characteristic surface markers, there is a small number of lymphocytes called "null" cells which lack traditional T- or B-cell markers. A group of lymphocytes called K cells or killer cells which participate in CMI reactions are included in this population of "null" cells. These cells are involved in a reaction called

antibody-dependent cell-mediated cytotoxicity (ADCC) which as the name implies, requires that target cells (e.g., tumor cells) be coated with specific IgG antibody. In addition to K cells, monocytes and neutrophils are also capable of ADCC activity against other targets, e.g., erythrocytes (36).

There are several soluble mediators, in addition to antibodies and lymphokines, which are involved in immune defense mechanisms. Two such mediators worthy of mention here are complement and interferon. Complement is a complex group of proteins which interact with each other following an antigen-antibody reaction. Once activated by a very specific sequence of interactions, complement is capable of producing lesions in the membrane of the target cell (e.g., bacterium, erythrocyte) bearing the antigen to which the antibody is bound. In addition to cytolytic activity complement also possesses chemotactic and immune adherence activity. Both of these latter functions serve to facilitate recruitment of phagocytic cells and phagocytosis of foreign material respectively (37).

Interferon is an antiviral substance that is released from cells following viral infection and serves as one of the first lines of defense against viral infections. It acts prophylactically by inducing other antiviral proteins that protect cells from viral infections by inhibiting virus-directed translation or transcription (38). In addition to its antiviral activity interferon also has immuno-modulating properties which include influencing B- and T-lymphocyte functions and stimulating natural killer cell activity and macrophage function (39).

Each element of the immune system plays a cooperative role in defending the host against infection and disease. A delicate balance exists wherein

the immune system not only protects the individual against foreign material but also is prevented from reacting to its own tissues. Alterations or dysfunctions in these elements or failures of homeostatic control may not only lessen the host's ability to combat infection and neoplasia, but also may lead to autoimmune disease. Immunopathological reactions may involve either humoral or cell mediated immune reactions. One group of immunopathological diseases in man and animals is immune complex disease. This group includes serum sickness, the Arthus reaction and nephrotoxic serum sickness. In general, these diseases are initiated by the formation of many small antigen-antibody complexes which localize in various tissues, resulting in acute inflammation and tissue pathology as immune complexes continue to be formed. Immune complex pathology may result as a consequence of a number of infectious diseases including bacterial, viral and parasitic infections (40).

Autoimmunity may be viewed as a breakdown of immune tolerance to autologous tissue either as a result of modification of "self" antigens or loss of suppressor cell regulation of immunoreactivity. Several autoimmune diseases of man develop spontaneously and can result from the production of autoantibodies and/or cellular immune reactions to tissues. Examples of autoimmune diseases in man are antiglomerular basement membrane nephritis, chronic thyroiditis (Hoshimoto's disease), autoimmune hemolytic anemia, systemic lupus erythematosus and idiopathic thrombocytopenic purpura (41).

Evaluation of environmental chemicals must include an examination of both potential immunosuppression and/or potentiation of immunopathological changes that may arise from the induction of an autoimmune state. Another consideration in immunotoxicology is whether a hypersensitivity is elicited

following exposure to the chemical. The following section deals with specific methodologies for assessing immune function.

B. Methodologies Used to Assess Immune Function

From the above discussion, it is apparent that the immune system consists of multiple cellular and soluble components which interact in a variety of ways to prevent infection and disease. Because of the complex nature of the immune defense system, there is no single test which can adequately assess the possible effects of a toxic substance on the developing immune system.

Various in vivo and in vitro tests which assess the functional capacity of individual components of the immune system and which help to delineate the site of immune dysfunction have been developed over the years (41-45). These include methods which assess immune functions directly, (e.g., protection against infectious diseases and antibody production against specific antigens) and those which assess immune functions indirectly, (e.g., the relative numbers of B- and T-lymphocytes and the mitogen-induced proliferation of lymphocytes in vitro). Obviously, one has much more latitude in the methods used to assess immune function in animals than in humans. Our knowledge of the immune defense system of humans has for the most part been gained by animal experimentation which has provided insights into the complex interactions of cells and cell products which comprise this system. In the following discussion, assays that are applicable to the detection of immunocompetence in humans will be highlighted with some references to animal work also included.

There are several methods for measuring soluble components of the immune system. An indirect assessment of a functionally intact humoral immune system can be accomplished by measuring immunoglobulin levels in

body fluids. Immunoelectrophoresis permits identification of the five major classes of immunoglobulins and is a good qualitative technique. However, for quantitation of class specific immunoglobulins, radial diffusion, automated immune precipitation, or electroimmunoassay ("rocket technique") are of greatest value. Using these techniques, immunoglobulin abnormalities such as immunodeficiencies and hypergammaglobulinemias can be detected (47).

Measurement of antibodies in human serum against specific antigens which result from infections by viral, bacterial or parasitic agents can be made using a variety of techniques. The physical and chemical composition of the antigen will dictate the type of assay. For example, nonsoluble antigens such as bacteria will agglutinate when mixed with antibodies against surface antigens. Soluble antigens which may include components of infectious agents such as cell wall and cell membrane molecules or intracellular components of bacteria, are detected by precipitation reactions. The actual quantitation of antibodies to specific antigens can be achieved by a variety of techniques which are in part dependent upon the eliciting antigen. These assays include but are not limited to the following: direct and indirect hemagglutination, complement fixation, radial diffusion, various radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), and viral neutralization assays (47).

The complement system mediates cytolysis and other reactions which contribute to inflammation and host defense. Evaluation of serum complement levels is principally used to detect individuals with inappropriate levels in order to determine the presence, extent and/or consequences of an immune reaction; to help assess the individuals response to a given therapy; and to detect individuals with inborn defects (48). Quantitation of serum

complement levels can be achieved by the following: 1) assays of hemolytic complement activity (i.e., dilution of serum required to lyse a known number of indicator cells under standard conditions); and 2) assays for individual complement proteins using radial immunodiffusion, radioimmunoassays or electroimmunodiffusion (48).

Measurement of the soluble elements interferons and lymphokines, unlike most of the assays for antibodies and complement which have as their basis physical and chemical interactions, involves the use of biological indicators. For example, quantitation of interferons is accomplished by measuring the ability of serum to: 1) reduce the number or size of viral plaques in cultured cells; 2) reduce the yield of viral particles, nucleic acid or viral associated enzymes; or 3) inhibit the generalized cytopathic effects of a virus in a monolayer cell culture (49). Lymphokines are soluble lymphocyte-derived mediators which may play a role in amplifying responses to antigens. These mediators, like interferons, require a biological endpoint for detection. Although these mediators are soluble, they are produced by T lymphocytes and thus are correlates of CMI. Lymphokines are generally detected by assaying for their presence in cell-free culture supernatants rather than by examining body fluids. Because of the great variety of lymphokines that may be produced following an immune response, a variety of assays are available for their detection (49).

Nonspecific indicators of lymphocyte immunocompetence include the enumeration of B- and T-lymphocytes in blood and lymphocyte transformation <u>in vitro</u>. Subpopulations of B lymphnocytes possess detectable membrane immunoglobulins (predominantly IgM and IgD in humans) and receptors for complement and the Fc region of IgG thereby permitting enumeration. Further, the erythrocytes

from several animal species bind to certain human lymphocytes forming rosettes which can be enumerated under a microscope. For example, mouse erythrocytes bind exclusively to human B lymphocytes, while sheep red blood cells (SRBC) form spontaneous rosettes with human T cells (50).

Human T lymphocyte subpopulations can also be studied using a variety of surface receptor and surface antigen markers. The advent of monoclonal antibody techniques has greatly enhanced the identification of specific T lymphocyte subpopulations. Enumeration of these B- and T-cell subsets along with other analyses of lymphocyte function, including lymphoproliferative responses to nonspecific mitogens and specific antigens, provides extremely useful insights for diagnosing and understanding immunodeficiency disorders, autoimmune diseases, tumor immunity and infectious disease immunity as well as malignant lymphocyte proliferation.

When lymphocytes are cultured in the presence of mitogens, large subpopulations of lymphocytes are selectively but nonspecifically stimulated to undergo transformation with attendant increases in protein, RNA and DNA synthesis. Lymphocyte transformation refers to an _in vitro_ correlate of an _in vivo_ proliferative reaction that occurs when an antigen interacts with specifically sensitized lymphocytes. Mitogen stimulated responses are generally quantitated by measuring the amount of radiolabeled thymidine incorporated into DNA by cultured lymphocytes. _In vitro_ lymphocyte transformation is clinically very useful as a sensitive indicator for assessing and monitoring inherited and acquired immunological deficiency states. The use of cell marker analysis in concert with the mitogen stimulated lymphoproliferative response helps to identify the subset(s) of lymphocytes associated with the patient's immunological defects (52).

T lymphocyte transformation following *in vitro* stimulation with T cell mitogens, such as phytohemagglutinin (PHA) and conconavalin A (Con A), serves as an indirect means of assessing cell mediated immune function. These nonspecific mitogens stimulate a large percentage of T lymphocytes with each mitogen having affinity for different subsets of T cells. Far fewer cells are stimulated, however, when using an antigen to which T cells are specifically sensitized (53). When such antigens are used a direct assessment of specific T lymphocyte proliferative potential is realized.

Another type of antigen stimulation in which T lymphocytes undergo proliferation is the response to foreign histocompatibility antigens, which are expressed on genetically unrelated cells. This reaction, called the mixed lymphocyte response (MLR), involves the coculture of lymphocytes from two individuals with unrelated histocompatibility antigens. These antigens, which have been found to be responsible for tissue rejection, are coded by genes in the major histocompatibility complex in humans, designated the HLA complex. The MLR is routinely used clinically in histocompatibility testing (i.e., tissue typing for allograph transplants) and as a test for immunocompetence of T cells, particularly in immunodeficiency diseases (53).

One of the best direct *in vivo* screening procedures for detecting aberrant cell-mediated immunocompetence in humans is delayed hypersensitivity skin testing. Delayed type hypersensitivity involves T lymphocyte-macrophage interactions as well as certain components of the inflammatory response. As a general rule, the development of a positive cutaneous reaction to one or more of several ubiquitous environmental antigens (e.g., purified protein derivative of tuberculin, *Candida*, streptokinase-streptodornase) or sensitization and subsequent cutaneous testing with a contact allergen (e.g.,

dinitrochlorobenzene) is indicative of intact cellular immunity (55).

In addition to the T cell enumeration and proliferative assays previously described, assessment of the ability of T cells to elaborate soluble mediators (lymphokines) which can alter the function of cells involved in CMI responses, can help define further the defect in cutaneous hypersensitivity. This includes assessment of the following lymphokines: migration inhibitory factor (MIF); leukocyte inhibitory factor; chemotactic factor; lymphocyte mitogenic or growth factor; lymphotoxin; and immune interferon (IFNγ). All of these factors have been shown to correlate with *in vivo* delayed hypersensitivity in humans (55).

There are several *in vitro* procedures for evaluating the functional capacity of monocytes (macrophages). These include measurement of phagocytic and bactericidal activity as well as chemotaxis. Although there are many macrophage functions, as well as numerous experimental systems employed to assess these functions there are no widely accepted standardized clinical assays currently employed. This situation has been suggested to result from the fact that there is a paucity of documented diseases or abnormalities of macrophage function (56). In contrast there are several clinically acceptable procedures for assessing neutrophilic granulocyte function because of the well-documented abnormalities of these cells (57).

Neutrophil dysfunction generally results in recurrent pyogenic infections. As with macrophages, the three basic functions that neutrophils must possess in order to eliminate certain invading bacteria are chemotaxis, phagocytosis and cytocidal activity. Routine clinical neutrophil function assays include assessment of chemotaxis, ingestion, degranulation and intracellular killing (58).

The approaches used to investigate immune dysfunction are undergoing rapid development. The methods given above do not therefore comprise a comprehensive list of standard clinical tests or of state-of-the-art methods. These methods serve as examples of the types of assays that are and have been employed clinically.

II. ONTOGENY OF THE IMMUNE SYSTEM

A. Fetal and Neonatal Immune Function Development

Human neonates can mount an immune response to many antigens and infectious agents. However, full protection from certain infectious agents is not realized until sometime after birth. Infectious disease in general is a major health problem in the young especially during the perinatal and infancy periods because both T- and B-lymphocytes are functionally immature at birth (59). Consequently, it is not surprising that the leading cause of human infant morbidity and mortality is neonatal septicemia (60).

Our understanding of the functional development of the immune system is far from complete. Nevertheless, there have been several studies which have significantly added to the growing body of evidence which has helped to delineate this developmental process. A great amount of our knowledge about immune ontogeny has come from animal studies. Direct comparison of immune development between human and animals, however, is complicated by many factors, not the least of which are the differences in developmental maturity at parturition. For example, animals with short gestation periods (e.g., mice, rats, rabbits and hamsters) have relatively immature immune systems at birth, with significant immune development occurring postpartum. On the other hand, in species with longer gestational periods (e.g., cows,

sheep and guinea pigs) the offspring have developed immunocompetence comparable to that of humans (61).

The following discussion outlines the ontogeny of the immune system in humans. It is generally accepted that human thymic epithelium appears about the sixth week of gestation with the evagination and migration of the third and fourth pharyngeal pouches. This epitheal anlage does not possess lymphocytes until about the ninth week of gestation (62) at which time hematopoietic stem cells from liver, spleen and perhaps bone marrow enter the thymus (63). Lymphocytes begin to appear in peripheral blood shortly after their appearance in the thymus and then in the dorsal mesogastrium, which will become the spleen (64). Lymphocytes which form rosettes with SRBC (T cells) are present in the thymus at about 9 weeks of gestation (65). PHA responsive lymphocytes in the fetal thymus are detectable by 12 weeks of gestation, with splenic and peripheral blood lymphocyte responsiveness to PHA detectable about four weeks later (66). T lymphocyte responses to Con A have been observed with thymus cells at 13-14 weeks of gestation and with spleen cells at about 18 weeks of gestation (67).

At 11-15 weeks of gestation, lymphocytes from human fetuses are capable of stimulating and responding to allogeneic lymphocytes in a mixed lymphocyte reaction (67). Despite this ability of lymphocytes to respond in an MLR at about 15 weeks of gestation, the lytic effector function of T cells to lyse allogeneic lymphocytes following sensitization in a cytotoxic T lymphocyte assay is not detectable even at 23 weeks of gestation. Furthermore, the capacity of sensitized T cells to lyse stimulator cells is not fully developed

(i.e., about half that found in adults) in full term newborns (69).

The development of other lymphocyte-mediated cytolytic activities appears during the latter period of gestation. For example, NK cell activity has been detected in peripheral blood at 28 weeks of gestation with full-term newborns displayed peripheral blood NK activity at about 60% that of adult levels (70). ADCC has been detected in cord blood at 28 weeks of gestation at levels comparable to that in adult peripheral blood (71). T cell helper function for the production of IgM formation following exposure to pokeweed mitogen (PWM) has been shown to reach adult levels by 6 months after birth (71).

A strong suppressor T cell function has been observed by many investigators in neonate (cord blood) lymphocytes. T cells from newborns inhibit the proliferative response of lymphocytes from adults in a mixed lymphocyte reaction (72). In addition, T cells from newborns strongly suppress pokeweed mitogen-induced immunoglobulin formation in lymphocytes from adults (73).

The development of B lymphocytes starts at about the same time as T lymphocytes. B lymphocytes (i.e., lymphocytes bearing IgM and IgG) are first found in the liver of human fetuses at about 10 weeks of gestation and in the spleen at about 12 weeks. IgD and IgA bearing cells are found at 12 weeks of gestation. Adult levels of B lymphocytes bearing membrane-bound Ig of all classes are reached by 14-15 weeks of gestation (59). However, neonatal B lymphocytes are functionally defective in their capacity to generate antibody producing cells <u>in vitro</u> compared with B cells from adults. In general, B lymphocytes of humans are inherently immature at birth. While a small number of IgM producing cells are detected, no IgG-

or IgA-producing cells can be identified. The ability of B cells to produce antibodies of the IgG and IgA classes increases with age with adult levels reached by 5 and 12 years of age, respectively (73).

It is not completely clear why these deficiencies exist. On the one hand, these deficiencies in IgG and IgA responses during the early period of life have been described as reflecting regulation by T cells (suppressor cells) as opposed to a deficiency in B cells. This high suppressor T cell activity has been reported to exist throughout infancy with decreasing intensity and disappears by about 2 years of age or later (73). On the other hand, a case has been made that deficient B cell function during the first several years of life is due to a B cell defect or immaturity rather than due to a negative influence by T suppressor cells or monocytes (59,74). In any event it has been postulated that the greater susceptibility of human newborns to certain bacterial infections is due to deficient B cell function particularly because of the delay in production of IgG and IgA antibodies (59). Perinatal humans appear to respond well to thymus dependent antigens primarily through adequate production of IgM. However, they respond poorly or not at all to antigens such as the polysaccharide antigens found on the cell walls of several infectious bacteria. These defective antibody responses significantly contribute to the increased susceptibility of neonatal humans to infections (74).

The immune responsiveness of neonatal humans appears to be influenced by their sensitization to antigens in utero and postnatally. There is ample evidence from laboratory studies to demonstrate that when animals are raised from birth in a germ-free environment there is a delay in lymphoid development (75). Germ-free animals have significantly reduced levels of

Ig, which is due to a 2-5 times lower rate of Ig synthesis. Synthesis of IgA and IgM is more affected than is IgG synthesis in a germ-free environment. The number of plasma cells in germ-free animals is about 10 times lower than in normal animals (59). These animals have a difficult time replacing maternal IgG, transported during gestation across the placenta, by a sufficient production of IgG of their own.

At birth nearly all of the IgG in the newborn human is of maternal origin. During the first 3 to 6 weeks of life, there is an exponential decrease in IgG which reaches its lowest level between 1.5 - 3 months of age. At this time IgG synthesis by the newborn begins but adult levels are not reached until 5-6 years of age. On the other hand, IgM levels rise rapidly during the early months of life with IgM at 75% of adult levels within 12 months of life. Only 20% of adult IgA levels are present at one year with adult level reached only after 10 years of age (76). The early increase in IgM levels in newborns parallels the development of natural antibodies to enterobacteria. Studies with newborn humans indicate that exposure to various nonpathogenic microorganisms or vaccines early in life gives rise to higher serum Ig titers than seen in controls (76). Further evidence comes from studies in which plasma cells and IgM production have been demonstrated in 28 week old human fetuses with congenital syphilis and toxoplasmosis. Elevated levels of IgM have been reported to occur in cord blood following congenital viral infections with herpes simplex, coxsackie and cytomegalovirus (76).

There is conflicting evidence regarding the Ig levels in premature and small-for-date newborns compared with normal babies. In general, however, infants of low birth weight have decreased IgG levels compared with normal

babies. This decrease in IgG levels at birth is in proportion to the degree of immaturity (76). Lower levels of IgM and IgA also have been reported in infants of low birth weight compared with full-size infants, with this difference persisting up to 6 months of age (76). A positive correlation exists between dietary protein intake and Ig production in human neonates. A significant decrease in serum IgG levels, with a trend toward lower IgA and IgM levels, has been reported in malnourished children without concurrent infection (77).

Passive immunity is provided to the newborn human by antibodies present in colostrum. Consequently, breast fed infants have greater resistance to many bacterial and viral infections. Secretory IgA is the main Ig in breast milk and plays an important role in protecting epithelial surfaces. Its presence along with other maternal antibodies to bacteria and viruses appears to function in the neonate's intestine to protect the epithelial surface against bacterial invasion and antigen uptake. Human colostrum contains IgA antibodies against bacterial pathogens of the neonate such as *E. coli*, staphylococci, and pneumococci and against polio virus (76).

Maternal antibodies can have a negative effect on neonatal immunocompetence by exerting a suppressive effect on neonatal antibody production. Inhibition of *de novo* antibody production in newborns with maternal antibodies has been shown to be related to the levels of maternal antibodies at the time of immunization (78). Suppression of the newborn IgM response by maternal IgG is more pronounced than by maternal IgM. This suppression by maternal antibodies can generally be overcome by the administration of a sufficient amount of antigen (76). Other soluble factors present in newborns, including bilirubin, also appear to exert a suppressive effect on antibody

production (60).

Functional immaturity of the phagocyte is a significant factor in the neonates impaired host defenses (60). The neonate's inflammatory response is insignificant by comparison to that of adults. This decrease in inflammatory responsiveness in the neonate is characterized by a higher proportion of functionally immature polymorphonuclear neutrophils (PMN) and fewer monocytes during the early hours of inflammation compared with infants 3-5 months of age. During the early period of inflammation in neonates, a large number of eosinophils are observed. This pattern of response is unique to this early period of life (79).

PMNs and monocytes from neonates (cord blood) are deficient in their chemotactic responses compared with cells from adults (60). Variable results have been obtained in studies on the bactericidal activity of phagocytes from newborn humans, but in the absence of unusual circumstances this activity appears to be normal (60).

Soluble mediators such as complement and interferon have been studied during fetal and neonatal life in humans. The complement component C3 has been reported to appear in fetal tissues as early as 6 weeks of gestation with C2 and C4 detected at 8 weeks of gestation. The appearance of complement in fetal tissue is due to synthesis by the fetus since little or no transfer of complement occurs across the placenta (80). In general, it appears that many of the components of complement, as well as the hemolytic activity of complement, are significantly lower in newborns compared to adult levels. Evidence suggests that deficiencies in the opsonizing capacity of complement in neonatal serum may contribute to the susceptibility of neonates to bacterial infections (60).

The *in vitro* induction of interferon production in lymphocytes obtained from fetal (8-17 weeks of gestation), newborn, child (1 month to 10 years) and adult tissue have been studied. The interferon titers obtained were in general lowest among the fetal tissue and highest among newborns and children. However, the data suggested that the interferon-producing capacity of human lymphocytes remains relatively constant throughout intrauterine and postnatal life (80).

The constantly changing immune capacity of the developing neonate coupled with the interaction of the immune system with other age related physiological functions makes it difficult to pinpoint specific times when immunologic maturity is reached. Much more work on the ontogeny of the immune system is necessary before a precise delineation of the functional capacity of lymphoid cells during the early stages of development is achieved.

B. Heritable Defects of the Immune System

The preceding discussion has presented evidence that the normal neonate displays many deficiencies in response to an infectious challenge which are related to incompletely developed host defense mechanisms. The neonate and young organism lack the full complement of mature immune inflammatory processes which are necessary for effectively combating infections. For example, group B streptococci infections are relatively easy to treat with antibiotics in normal adults or older children. However, these bacteria have been shown to be one of the most common etiologic agents isolated from infants with septicemia and/or meningitis (60). An interesting clinical observation is the fact that patients with immunodeficiency disorders also have a high incidence of infections with organisms that in normal subjects are self-limiting or readily treatable with drug therapy. Furthermore, the

clinical picture of immunodeficiency is characteristically expressed as a marked susceptibility to opportunistic and pathogenic organisms similar to that just described for neonates.

Immunologic deficiencies make up a diverse group of disorders which can be classified in a number of ways. One method of classification describes the disorders as primary or secondary (8). Primary immunodeficiencies generally are hereditary and are manifested early in life as repeated infections with microorganisms which produce mild infections in normal children and adults. Primary immunodeficiencies are generally subdivided into disorders of the B cell system, the T cell system or both B- and T-cells. Secondary immunodeficiencies are disorders in which the initiating factor is not part of, or restricted to the immune defense system. These deficiencies occur more frequently than the primary disorders and occur in older individuals. Examples of secondary immunodeficiencies include those which arise from certain viral infections, enzyme deficiencies (e.g., adenosine deaminase and nucleoside phosphorylase deficiency), the use of immunosuppressive agents, and protein-calorie malnutrition (8). Important clues as to the particular defect in the immune system can be provided by the type(s) of infection(s) observed in the patient. For example, recurrent infections with encapsulated pyogenic bacteria might indicate either a defect in the B cell system, with a failure to produce antibodies, a defect in phagocytosis or a defect in the complement system. A defect in the T cell system, on the other hand, might give rise to the development of infections by intracellular parasites such as bacteria, viruses and fungi. Immune defects are clinically detected and delineated using the various methods described above (Section I.B.).

The essential feature of a heritable immunodeficiency is the inability to mount a humoral or cell-mediated response because of a developmental abnormality. Four examples of primary immunodeficiencies are the following: 1) x-linked agammaglobulinemia (Burton's Disease); 2) thymic aplasia (DiGeorge's syndrome); 3) ataxia telangiectasia; and 4) severe combined immunodeficiency disease.

X-linked agammaglobulinemia is a sex-linked recessive disorder (manifested in males) that is essentially a defect in the B cell system (82). This disease is characterized by a deficiency in all immunoglobulins except IgE, a lack of plasma cells in lymphoid tissues, and recurrent infections with encapsulated bacteria and certain viruses. These patients, however, have a normal thymus and are able to develop normal cell-mediated immune responses (8).

In contrast, DiGeorge's syndrome is a basic defect that involves the T cell system. This congenital abnormality results from the absence or poor development of the thymus which is believed to result from an undefined intrauterine insult affecting the third and fourth pharyngeal pouches. Clinically, patients with DiGeorge's syndrome have depressed cell-mediated immune responses and are susceptible to infections with intracellular microorganisms. There is depletion of lymphocytes in the thymic-dependent areas of the lymphoid tissue; however, B cell functions are generally normal with usually normal serum immunoglobulin levels present (83).

Ataxia telangiectasia is an autosomal recessive disorder that is clinically associated with recurrent sinopulmonary infections as well as with an increased incidence of lymphoreticular tumors. In most patients, abnormalities of both the B- and T-cell systems are present. This disease is characterized

by a thymus which is usually small and lacks cortical and medullary organization. Thymic-dependent lymphoid tissues are usually depleted of lymphocytes, and cell-mediated immunity is generally depressed. Deficiencies in IgA and/or IgE are the most prominent examples of B cell system involvement. In many patients, an intrinsic defect in the B cell system results in the failure of IgA bearing cells to develop into IgA producing plasma cells (84).

Severe combined immunodeficiency disease (SCID) includes a heterogeneous group of diseases. In general, SCID patients have abnormal numbers of B- and T-cells, decreased antibody (humoral) responses, and depressed cell-mediated immunity (8). SCID is the most severe and most clinically characteristic form of immunodeficiency which occurs during the neonatal period. Symptoms generally occur during the first month of life. Without appropriate treatment, death is inevitable within 1 to 2 years of age (60). This disorder may be inherited either as an autosomal recessive form (Swiss type) or as an x-linked form. In Swiss type SCID, lymphoid cells fail to develop and T- and B-cells are greatly diminished. Reticular dysgenesis, which arises from a defect at the level of the hematopoietic stem cells resulting in the virtual absence of B cells, T cells and neutrophils, is the most severe form of SCID (8).

Another type of SCID results from enzyme deficiencies. Adenosine deaminase (ADA) deficiency has been associated with both T- and B-cell defects. The enzyme deficiency results in the accumulation of deoxy adenosine triphosphate (d-ATP) which is especially toxic to lymphocytes (85). Another enzyme deficiency involves the enzyme purine nucleoside phosphorylase (PNP), which is associated with impaired T cell function but normal B cell function (86). Both ADA and PNP deficiencies are inherited as autosomal recessive traits.

Another form of SCID is Wiskott-Aldrich syndrome which is a sex-linked recessive disorder that is characterized by recurrent infections, variable T- and B-cell abnormalities, thrombocytopenia and eczema. Serum IgM levels are usually low with elevated serum IgA and IgE (87).

In addition to the above primary immunodeficiencies which affect lymphoid elements, there are several intrinsic defects in phagocyte function. In chronic granulomatous disease (CGD), both monocytes and granulocytes are deficient in NADH- or NADPH-oxidase or NADPH-reductase. This disease is inherited as an x-linked disorder which appears during the first 2 years of life. As a result of these enzymetic deficiencies, the intracellular metabolism of phagocytic cells is altered, resulting in decreased intracellular killing of certain bacteria and fungi (88).

Both granulocytes and monocytes have defective microbicidal and chemotactic activity in patients with Chediak-Higashi syndrome. This disease is a rare multisystem autosomal recessive disorder (8). Patients with Chediak-Higashi syndrome also have defects in natural killer cell activity (89).

Defects in the complement system also exist. Patients with deficiencies in some of the complement components have increased susceptibility to systemic lupus erythematosis as well as other collagen vascular diseases (90). Deficiencies in other complement components may lead to pyogenic infections (8).

The consequence of all of these disorders is frequent and persistent infection. In addition, certain disorders can lead to increased risk of malignancy. Heritable defects in immune system development are difficult or impossible to correct. In general, impaired T cell immunity is more serious than a deficiency in B cell immunity because it has an ultimate affect not only on CMI but also on humoral immunity (8).

III. ENVIRONMENTALLY INDUCED DEFECTS IN THE DEVELOPMENT OF IMMUNOCOMPETENCE

Intrinsic primary immunodeficiencies, as described above, offer excellent examples of the potential consequences of extrinsic or environmentally induced immunosuppression. The effects of various immunosuppressive drugs and ionizing radiation on the immune system are well known. The extent of adverse effects on the immune system resulting from exposure of adult animals to a wide variety of environmental chemicals has only recently begun to be appreciated (4-7). Even less is known about the magnitude of the immunological effects resulting from fetal and neonatal exposure to a variety of xenobiotics including drugs, food additives and environmental chemicals. One would expect that disruption of the normal sequence of events in the development of the immune system by extrinsic factors would result in altered immunocompetence. This has been shown to be the case in a number of animal studies which have recently been reviewed by Roberts and Chapman (12) and Schmidt (91). As for humans, the potential exists that exposure to environmental chemicals, drugs, or food contaminants during gestation and/or early in life could affect the immune status of the young. The effects of malnutrition on the immunocompetence of young children attests to this hypothesis (77).

Table 1 summarizes the effects of a variety of chemical, physical and biological factors which have been shown to affect the developing immune system. All but one of these studies (77) have been performed using experimental animals. Of particular interest is the fact that agents which are capable of altering immunocompetence in the adult generally are more effective when administered perinatally (5). Agents such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (103-105), corticosteroids (98-100), busulfan (101), lead (109,110),

Table 1. Developmental Immunotoxicology

Agent	Species	Period of Exposure	Effects	Ref.
Dietary protein deprivation	Human	12-36 months of age	Decrease in IgG levels, trend toward lower IgA and IgM levels, decrease in cutaneous hypersensitivity and PHA response, reduced response to S. typhi vaccine	77
	Rat	Day 1-22 of gestation	Fetal growth retardation and delayed thymic development	92
	Mouse	4-6 weeks of age	Decreased T-cells, decreased response to PHA and MLR, retardation of immune maturation	93
Zn deficiency	Mouse	Lactating mice	Pups showed retardation of thymus and spleen development, depression of mitogen response to T-cell mitogens, absence of detectable IgM and IgA	
	Mouse	Day 7-21 of gestation	Reduced antibody response to SRBC and depressed serum Ig for up to three generations	97
Corticosteroids	Rat	Day 8-20 of gestation	Fetal growth retardation, delayed thymic development	98
	Rat	1-day-old	Reduced antibody response to SRBC	99
	Mouse	1-day old	Thymic atrophy	100
Busulfan (1,4-butanediol dimethane-sulfonate)	Mouse	Day 1-18 of gestation	Growth retardation, hypoplasia of the thymus	101
Benzo[a]pyrene	Mouse	Day 11-13 or 16-18 of gestation	Reduced antibody response to SRBC, increased tumor incidence	102
TCDD (2,3,7,8-tetra-chlorodibenzo-p-dioxin)	Mouse	Day 14 of gestation and 1,7 and 14 days of age	Depressed colony formation of macrophage-granulocyte progenitor cells and pleuri-potent stem cells, reduced resistance to bacteria and tumors, depressed response to PHA, Con A and LPS	103

Table 1. Developmental Immunotoxicology (Continued)

Agent	Species	Period of Exposure	Effects	Ref.
TCDD	Rat	Day 18 of gestation and 1,7 and 14 days of age	Thymic atrophy, reduced response to PHA and Con A and reduced DTH	104
	Rat	Days 11 and 18 of gestation, and 4,11 and 18 days of age	Thymic atrophy, reduced response to PHA, reduced graft vs host response	105
	Mouse	Days 14 and 17 of gestation, and 1,8 and 15 days of age	Delayed skin graft rejection, reduced GVH response	105
HCB (hexachlorobenzene)	Rat	Through pregnancy, lactation and 5 weeks of age	Reduced resistance to bacteria and parasites, enhanced humoral immunity	106
PBB (polybrominated biphenyl)	Pig	Last 8 weeks of gestation and during lactation	Reduced response to B-cell mitogen	107
PCB (polychlorinated biphenyl)	Rabbit	Four weeks prior to mating and then through gestation and lactation	Reduced contact sensitivity to dinitrofluorobenzene	108
Lead	Rat	At weaning through mating, gestation, lactation and then postnatal period	Reduced response to SRBC, thymic atrophy, reduced response to PHA and Con A, reduced DTH response	109,110
Nonionizing electromagnetic radiation (thermogenic levels)	Rat	Day 6 of gestation through 41 days of age	Alterations in T- and B-cell mitogen responses	111,112
	Mouse	Day 6-18 of gestation	Reduced natural killer cell activity	113
DES (diethyl-stilbestrol)	Mouse	Day 16 of gestation	Thymic weight reduction, reduced DTH in females, reduced response to Con A and LPS	114
	Mouse	Days 1-5 of age (females only)	Reduced response to Con A and LPS, reduced antibody response to SRBC and LPS, reduced helper T-cells, reduced NK activity	115,117

Table 1. Developmental Immunotoxicology (Continued)

Agent	Species	Period of Exposure	Effects	Ref.
Testosterone	Chicken	Day 5 of incubation	Inhibition of development of the bursa of Fabricius, reduced antibody response	118,119
Corn oil	Rat	Day 15-19 of gestation	Reduced response to Con A and PHA	120
Chlordane	Mouse	Throughout gestation	Reduced contact hypersensitivity to oxazolone	121

diethylstilbestrol (114-117), thermogenic levels of nonionizing radiation (111,112) and benzo[a]pyrene (102) affect the developing immune system more profoundly than the mature immune system. Other agents have been found which impair immunocompetence only when administered prenatally. For example, testosterone which essentially does not affect the immune response of adult animals, is extremely effective in bursectomizing embryonic chickens and thereby eliminating B cell responses (118,119). Another example is corn oil, which when given in utero reduces the response to T-cell mitogens (120) but has no effect on adults (122).

While this author is unaware of any clinical studies in which the effects of environmental agents on the developing immune system of humans has been monitored, it is not difficult to envision the potential for environmental agents to cause developmental immunotoxic effects in humans based on the animal studies summarized in Table 1.

IV. SUMMARY

The immune system is composed of various cells and cell products which together provide the individual with protection from infections and neoplasia. There is a good deal of redundancy within the system such that subtle perturbation can be compensated for so that homeostasis is maintained. However, when alterations in a given component of the immune system are of sufficient magnitude, the health and well being of the individual can be severely compromised. Such alterations, whether they be intrinsic (e.g., immunodeficiency diseases) or extrinsic (e.g., environmental toxicants), can lead to a decrease in the quality of life. This diminution in the quality of life can be due to debilitation resulting from recurrent infections, development of immunopathologic diseases due to impairment of immune regulatory functions (e.g.,

antoimmune disease, hypersensitivity), or development of neoplasia. At the extreme, death can result consequent to these complications which have at their root impaired immune function.

The immune system is obviously an organ system critical for survival. However, unlike many other critical organ systems which maintain continuous function, the immune system can be considered to remain dormant until the host is challenged with an infectious agent or malignant growth (12). When the host is challenged a rapid and directed response is required. Because of the unique properties of the immune defense system measurement of the integrity or potential of its numerous components is more complex than for most other critical organ systems. Consequently, in order to evaluate adequately potential defects in this system, methodologies which assess the functional integrity of these various components are necessary. These methodologies have been and continue to be developed and validated so that they can be accurately applied to delineate an immune function defect. These same techniques have also been used to chart the milestones in the development of the immune system.

Studies of immune ontogenesis have revealed that even at birth, mammals do not possess fully competent immune defense systems. While humans have more developed immune systems than other mammals at birth, the human infant is still immunologically immature. The infant (i.e. < 1 year) is particularly vulnerable to a number of infectious diseases which at times are difficult to diagnose because the typical clinical symptoms that appear in older children do not appear in the infant due to its immature immune defense system (60). Perturbation in the immune defense system of the newborn by xenobiotics can be of significant consequence because of the newborn's yet

incomplete reserve capacity. In utero exposure to xenobiotics such as environmental toxicants, drugs and physical agents which induce immune defects can be of even greater consequence. The examples from animal studies cited above in which toxic agents have altered the developing immune system provide evidence of the potential problem for perinatal human exposure to these and other agents. In addition, the examples presented of inherited immune dysfunctions offer insights into the potential consequences to the quality of life that might result from a toxic agent-induced impairment of immune development.

Developmental immunotoxicology is not as yet a bona fide discipline in the true sense of the word. Yet more and more researchers are realizing the tremendous potential of its application to the broader disciplines of immunology, toxicology, teratology and developmental biology. We have at our disposal the tools of these various disciplines which can be applied to assess the impact of environmental agents on the developing immune system. By employing standardized and validated methodologies to identify developmental immunotoxicants, in concert with clinical and epidemiological studies, preventive measures can be taken to avoid perinatal exposure of this high risk population to the offending agents.

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